THE EFFECT OF ALKALIS ON THE STABILITY OF KERATINS[1]

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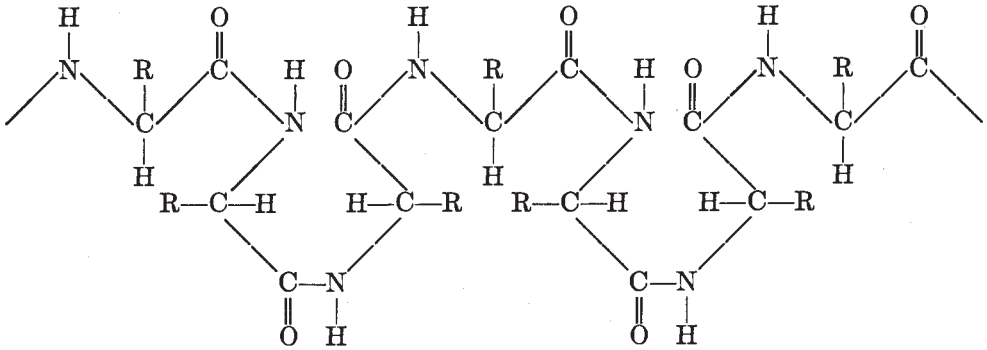
(Received for publication December 13, 1941)

In a series of three studies previously reported in THIS JOURNAL (1, 2, 3), various aspects of the investigation of nails and nail changes were submitted. The last article dealt with brittleness of the nails and its possible causative factors. The most important avenue for investigation appeared, from the broad surveys on the occurrence of nail changes, to point to the effect of alkalis on keratin. A search of the literature revealed many inconsistencies regarding the biochemistry of the nail. Little, if anything, was to be found with reference to the reactions of keratin derived from human sources.

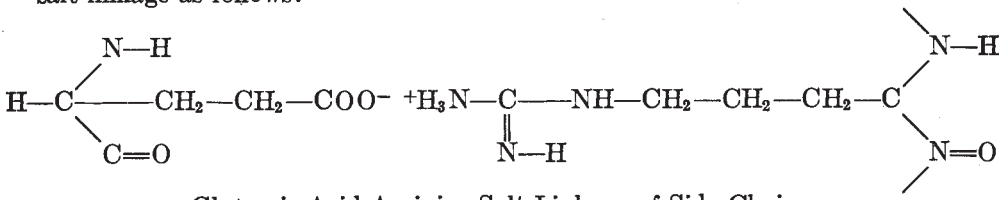
The first real contribution to the better understanding of keratin was made by Unna (4) and Rothman and Schaaf (5). The reported values for cystine, tryptophane and tyrosine varied greatly as did the values for ash content and fat.

Research on the reactions of wool keratin[2] indicated a possible parallel to nail keratin. A complete hydrolysis of wool shows that it is a protein composed of a large number of amino acids of which the principal ones are tyrosine, cystine, leucine, tryptophane, arginine and glutamic acid. These and other amino acids are combined in long chains of peptide linkage and these chains are thought to be folded in some manner as indicated below:



Folded Peptide Chain

These folded chains are further thought to be connected by side chains with a salt linkage as follows:

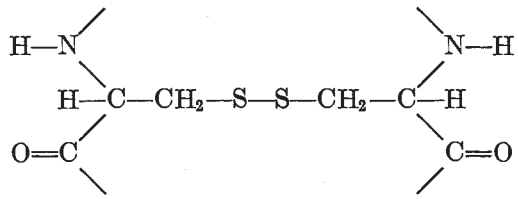


Glutamic Acid-Arginine Salt Linkage of Side Chains

[1] Material used by B. Chiego in thesis for Degree of Doctor of Science, McKinley-Roosevelt Foundation, Chicago, Ill.

[2] The authors wish to express their gratitude for much kindly assistance given them by Dr. Jacinto Steinhardt of the U. S. Bureau of Standards.

The folded chains are also thought to be connected by side chains with a cystine linkage:



Cystine Linkage of Side Chains

The cystine linkage seems to be the most important of these side chains. In cases of chemical attack on wool keratin the points affected, in order of greatest sensitivity, are apparently, first, the disulfide linkage and then the peptide linkages.

The types of chemical treatment to which wool keratin is exposed and which may cause damage are:

1. Acids
2. Alkalis
3. Hot water or steam
4. Reducing and oxidizing agents
5. Chlorination or bromination (nonshrinkage processes)

The reactions of keratin which may serve to explain the above may be represented as follows:

1. *Effect of steam* (short exposure).

a. $R—CH_2—S—S—CH_2—R + HOH$

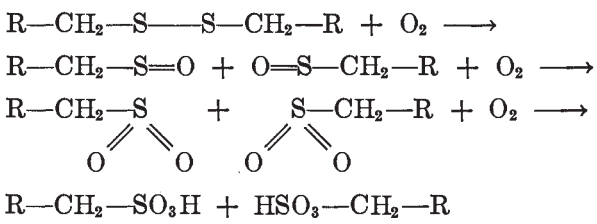
b. $R—CH_2—S—OH + H—S—CH_2—R$

This sulfenic acid derivative may react with another amino group to yield $R—CH_2—S—NH—R$

This group gives the the lead acetate test

2. *Effect of Alkalis.* Alkalis speed up the hydrolysis represented in 1 above. The first reaction is one of peptization up to pH 9 (swelling of the keratin complex—"quellung"). Above pH 9.2, and depending upon the concentration, time of contact, and temperature, rapid hydrolysis occurs. Speakman (6) has determined that rapid hydrolysis begins at pH 9.2 (this is about the pH of of a 2 per cent borax solution). In the case of caustic soda or potash the hydrolysis goes more to completion with the formation of soluble sodium salts or the amino acids and small amounts of soluble sulfides are formed. In the case of alkalis, therefore, the hydrolysis of keratin occurs irreversibly above pH 9.2 with great destructive effect.

3. *Oxidizing agents.* These affect the cystine portion of the keratin complex at the disulfide linkage in various stages as follows:



4. *Aldehydes.* Aldehydes react with the amino groups of the amino acids as follows:

This reaction takes place at room temperature destroying the NH_2 groups, thus reducing plasticity.

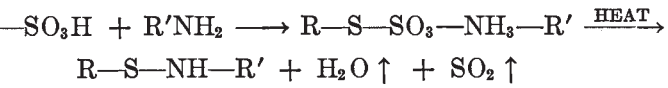
5. *Sulfites.* This is important because the reaction is made use of in the permanent waving of hair. The reaction may be represented as

a. Hydrolysis

b. Coupling of the residues

(a)

(b)



The above represent in brief some of the reactions of keratin as deduced from the behavior of wool keratin in processing.

ACID BASE BINDING CAPACITY OF KERATIN

In addition to the above reactions, an important characteristic of keratin is its ability to bind acids and bases. This is so even where extreme dilutions (0.02 M) are used with low temperatures (0°C.).

Steinhardt and Harris (9) state that the nature of the dependence on pH of the degree of combination of acid and base by such insoluble proteins as keratin (wool), silk and collagen differs in certain characteristic ways from that of soluble proteins. Examination of measurements reported by Speakman and Hirst (7), Lloyd and Bidder (8), and others, leads to the conclusion that the titration curves of these three proteins determined in the absence of salt, are distinguished from those of all soluble proteins, so far studied (except those, such as hemoglobin, in which irreversible effects occur) under the same conditions in three principal respects:

1. They show a region of little or no acid-or-base-binding capacity, more or less symmetrically placed about the point of neutrality. This is true even when, as in the case of wool keratin and silk, they contain appreciable quantities of histidine which, because of its imidazole group, should function as a buffer in this region.

2. The amount of acid and base bound increases sharply as the extremes of the pH scale are approached and reach their respective maximum values at about the

same pH as in the case of soluble proteins. Thus, the titration curves are steeper than those of soluble proteins, or of soluble polybasic acids in general.

3. The pH values at which half of the maximum amount of acid or base is combined (a convenient measure of the position of the curve and, in simple substances, directly related to the acid strengths of the groups titrated), are shifted considerably toward the two extremes of the pH scale. If these curves are regarded as resulting from the titration of many groups which possess identical dissociation constants this difference in the position of the midpoints would represent a fifty-fold increase in the acid strength of the insoluble protein, although its dissociating groups are presumed to be the same as those present in proteins which may be titrated in the dissolved state.

Steinhardt and Harris (9) have reported an excellent study of the base binding capacity of wool keratin. These authors used the method of Harris and Rutherford (10) except for the substitution of brom cresol purple for methyl red as indicator, and the use of nesslerization for the determination of the amount of dissolved protein. Additional aliquots were used for immediate measurement of the pH at the temperature of the experiment.

The fact that wool keratin binds appreciable amounts of base is indicated by the plotted line II on graph 4.

Hence it is apparent that the keratin complex must combine with or absorb equivalent quantities of both positive and negative ions. Thus, its ability to combine with OH ions is limited by the simultaneous availability of H ions. In the case of HCl the same holds in that the ability to combine with hydrogen ions is limited also by the simultaneous availability of chloride ions.

EXPERIMENTAL—1

During the course of studies on the behavior of wool keratin it was thought advisable to check the results with the keratin of human hair. Because cystine occurs plentifully in hair, wool and horn it was suspected that the occurrence is probably related to the growth promoting effect of cysteine, which on oxidation gives cystine, the ordinary change from a sulfhydryl compound to a disulfide.

The activity of the sulfur in cystine and cystine containing proteins has always presented a complex problem. In the present paper, the interest of the authors has been centered on this amino acid because of the experimental results obtained in working with human hair for the preparation of cystine.

It was found that the yields of cystine obtained from various batches of human hair was in no way constant.

Determinations of cystine by the method of Gortner and Hoffman (14) demonstrated that when the hair is previously allowed to stand over night in a solution of dilute (0.5 per cent) Na_2CO_3 it was found that no cystine could be recovered. Boiling dilute Na_2CO_3 in contact with hair for one to one and one-half hours had the same effect.

It has also been observed that on treatment of human hair with standard alkali solutions the titratable alkalinity decreased proportionately with temperature and time of exposure as it does in the reported observations on wool.

EXPERIMENTAL—2

The macroscopically normal nail clippings used in these studies were prepared by thoroughly agitating in cold distilled water and centrifuging out all dirt clippings observed under the microscope were found to be free from adherent foreign matter. This took 1.5 to 2.0 hours utilizing a high speed stirrer fixed with a specially constructed hard bristle brush. All small particles were discarded and only pieces of whole nail substance averaging 7 mm. in length by 1.5 mm. by 0.75 mm. were used. The nail clippings thus prepared were conditioned at 60 per cent relative humidity at 37°C. The moisture content was found to average 11.3 per cent. The nails were dried to constant weight over concentrated sulfuric acid in vacuo. The ash determined as oxides was found to average 0.0284 per cent based on the original nail substance containing 11.3 per cent moisture.

Weighed portions of nail clippings were then exposed to N/10 NaOH and approximately 1 per cent NaOH for 20 hours at 37°C. The alkali solutions were carbonate and sulfate free.

The time of 20 hours was adopted because it was found that it required 18 to 20 hours for equilibrium to be attained.[3]

At the end of the 20 hour period the liquids were freed from the nail by centrifuging and aliquots were taken for titration of bound alkali in each case. Check runs proved the correctness of Dr. Steinhardt's suggestion for an exposure of 20 hours to the solutions.

Aliquot samples were also taken for the determination of split S—S bonds. Ten cubic centimeters samples were oxidized with bromine on a water bath for 1 to 1.5 hours and the sulfate was precipitated with barium hydroxide, collected on a sintered glass crucible washed with concentrated HCl, distilled water and isopropyl alcohol, dried to constant weight and weighed. The per cent sulfur was calculated and was referred to the per cent cleavage of the disulfide bonds.

The calculations for per cent S—S bonds cleaved were based on our own determination of cystine which is based upon the modification suggested by M. X. Sullivan (15). This method was found convenient because of the small amounts necessary and the accuracy of rechecks.

Results: The average results of four determinations on nail clippings showed 11.97 per cent cystine which is equal to 3.2 per cent sulfur. This figure was taken as a standard for all subsequent calculations.

The drop in available cystine, under the conditions of this experiment, serves to indicate that alkalis play a direct role in the destruction of the disulfide bond in the cystine portion of the keratin complex.

The effect produced on human hair has been repeatedly observed in wool keratin.

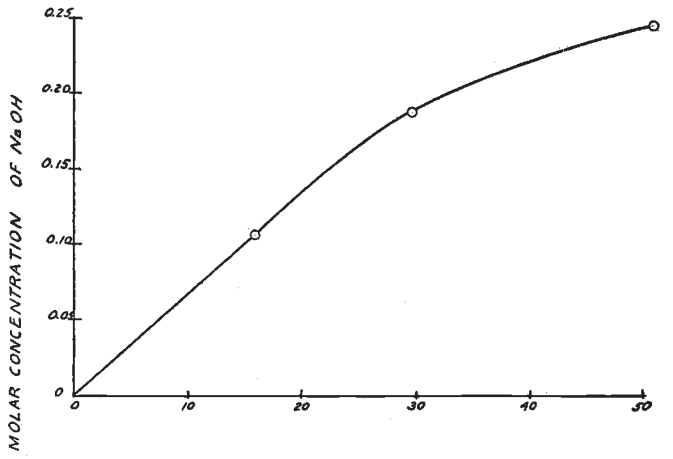
It is, therefore, apparent that since the cystine could not be recovered after the exposure of human hair to alkali for the temperature and time indicated, an

[3] Personal communication from Dr. J. Steinhardt of U. S. Bureau of Standards.

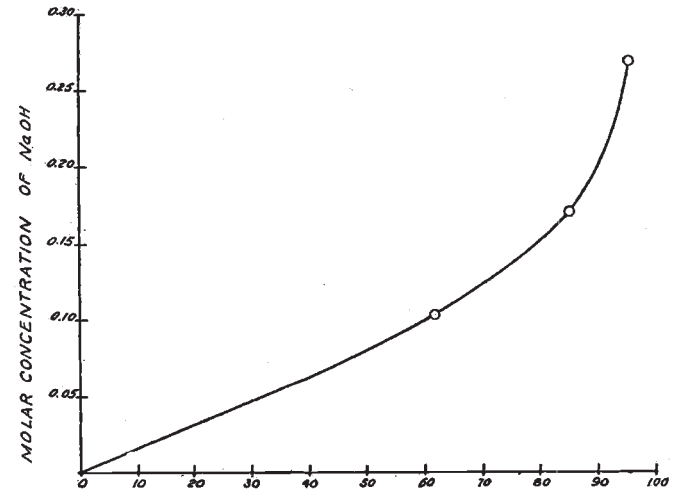
investigation of the degree of stability of the disulfide linkage is all that is necessary to determine the destructive effect.

DISCUSSION

The results of the experiments reported herein may be briefly summarized by the following graphs:



GRAPH 1



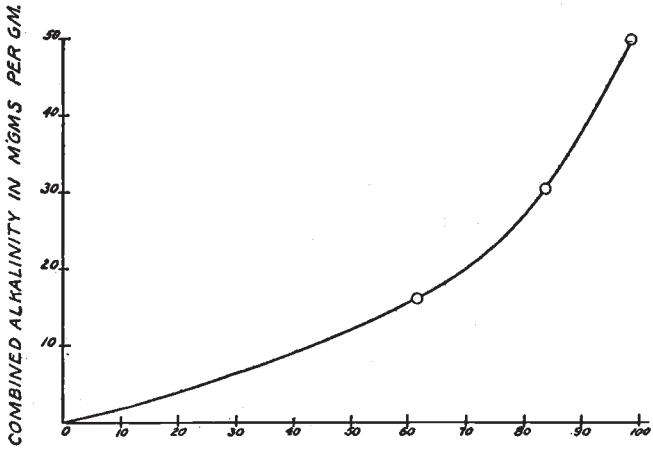
GRAPH 2

Graph 1: This shows the molar concentration plotted against the combined alkalinity per gram of anhydrous nail keratin.

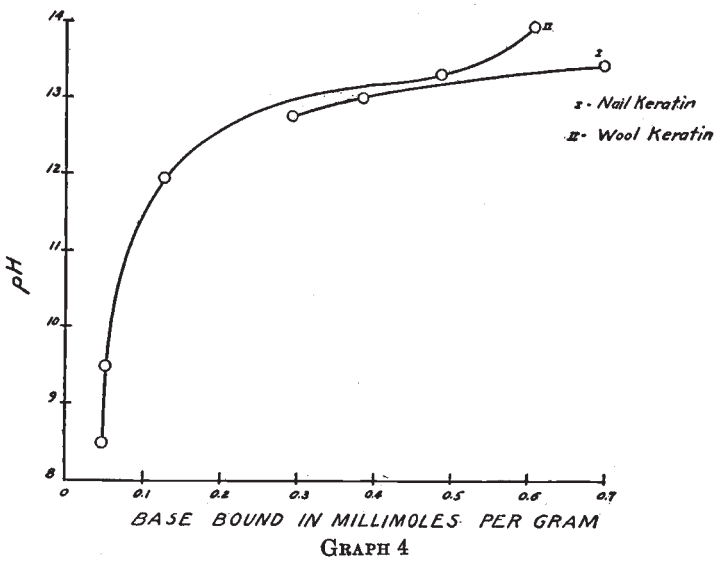
Graph 2: This shows the extent of S—S decomposition plotted against the molar concentration of sodium hydroxide.

Graph 3: This describes the extent of S—S cleavage plotted against the combined alkalinity based on anhydrous nail substance.

Graph 4: In this graph the logarithmic expression of curve number 1 is compared to the curve derived from the values reported by Steinhardt and Harris (9). The transposition of the curves probably is caused by the following: 1) Temperature of reaction—Our reactions were conducted at body temperature while those of Steinhardt and Harris were conducted at 0°C. 2) Our findings were not corrected for decomposition at 37°C.



GRAPH 3



GRAPH 4

Since it has been shown that the cleavage of the S—S bond of cystine makes available only one half the sulfur content for precipitation as the sulfate, the values obtained experimentally need only to be doubled to obtain the per cent of cystine decomposed. This refers, of course, only to the disruption of the disulfide linkages.

It is of real interst to note that the behavior of nail substance and human hair closely parallel the behavior and reactions of the keratin of wool. The results indicate a similarity in behavior which is related to the cystine content of both types of keratin.

The reported average values for the cystine content of the keratin of wool vary between 12.9 and 15.27 per cent. The values for the cystine content of nail substance have been variously reported as 19.26 per cent by Moerner (12) and 10.39 per cent by Langecker (13). The most consistent results were obtained by Klauder (11) whose results yield an average of 12.15 per cent cystine. The value obtained by the authors (11.97 per cent) closely approximates the findings of Klauder and his associates.

The results of the decomposition studies show that clippings of normal nails, when exposed to alkali concentrations of from 0.1014 N to 0.2468 N show 61.4 per cent and 97.6 per cent of the disulfide linkages of the cystine portion of the keratin complex destroyed upon twenty hours contact at body temperature.

CLINICAL IMPLICATION

It will be recalled that the destructive effect of alkalies on nail keratin was suspected from a survey on the incidence of brittleness among workers in various fields (3). The fact that parallel destructive effects are found for both the keratin of wool and the keratin of the hair and nails is of fundamental importance from the dermatologic viewpoint. When one considers that alkalis, whether in the form of soaps or more alkaline cleansers, are the most frequently and universally used daily, the suspicion naturally follows that alkalinity may be a factor in predisposing the skin to sensitizing reactions.

The commonly observed nail change, Fragilitas Unguium, is manifested by a fraying of the free edge of the nail and a peeling of the upper layer of the nail plate. This so-called brittleness of the nails is becoming fairly common. The condition can be readily and easily explained by the reactions of keratin reported in this paper.

Considering the surfaces exposed to an alkali contactant, it is possible to predict exactly the changes which are found in cases of brittleness.

On the suggestion of Dr. S. Rothman[4] we intend to study the swelling of normal and diseased nails at different pH levels.

The authors attempted to compare the treated nail clippings with normal nail clippings (untreated) by testing them for breaking load capacities. The tests were discarded because of the extreme brittleness of the clippings which had been exposed to alkali.

[4] Personal communication: The authors wish to express their gratitude for the kindly assistance given them by Dr. Stephen Rothman.

SUMMARY AND CONCLUSIONS

1. The cystine portion of the keratin complex of human hair is as readily decomposed by alkalis at the disulfide bond as is the cystine of the keratin of wool.

2. The alkali-binding effect of nail substance closely approximates that of wool.

3. The cleavage of the S—S bonds in the cystine portion of the keratin of nails is readily accomplished by alkalis.

4. Large amounts of keratin are decomposed at the S—S bond of cystine by alkalis at 37°C. The per cent decomposition was found to be 61.4 per cent and 97.6 per cent respectively on 20 hours exposure.

5. The alkali degradation of keratin is undoubtedly a primary factor in the causation of brittleness of the nails.

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